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ON THE OCCURRENCE OF PYCNIA AND AECIA IN CERTAIN RUST FUNGI[1]

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Evidence secured by experimentation in the greenhouse has demonstrated that *Puccinia helianthi* Schw. and *P. graminis* Pers. are heterothallic; that the majority, at least, of pustules derived from infections by single sporidia of these rusts never produce aecia;[2] and that when pycniospore-containing nectar of such pustules is transferred from one to another, so that a thorough mixing is effected, aecia appear in a few days.[3] A few additional observations and experiments bearing on the problem of sex in rust fungi are set forth in this paper.

For the sake of convenience in what follows, pustules of monosporidial origin are designated as "simple" pustules, and those of bisporidial origin as "compound" pustules.

EVIDENCE OF HETEROTHALLISM IN PUCCINIA GRAMINIS

It was intimated in one of the previous papers[3] that *Puccinia graminis* is heterothallic, but, as that paper treated specifically of the function of the pycnia of rusts, no data were furnished to show the relative numbers of compound pustules and simple pustules which did, and which did not, produce aecia. A statement concerning this point will first be made.

Both simple and compound pustules of *P. graminis* were obtained on young leaves of barberry (*Berberis vulgaris*) by sowing sparsely the sporidia of germinating teliospores over the surface of the leaves. Figure 1 shows a barberry leaf which was photographed 20 days after it was inoculated. A compound pustule bearing aecia appears on the left-hand side of the midrib. On the right-hand side are three simple pustules in which no aecia have developed. The pitted appearance of these three pustules is due to the formation of haploid mycelial wefts which develop in the simple pustules just underneath the epidermis, and simulate in general contour that of a young aecium. They never break through the epidermis, however, and rarely, if ever, of themselves produce aeciospores. This point will be discussed in a subsequent paper.

[1] Contribution from the Division of Botany, Dominion Experimental Farms Branch, Ottawa.

[2] Craigie, J. H. Experiments on Sex in Rust Fungi. Nature 120: 116–117. 1927.

[3] Craigie, J. H. Discovery of the function of the pycnia of the rust fungi. Nature 120: 765–767. 1927.

The pustules first appeared as tiny dots. As soon as they became evident, the position on the leaf of each individual pustule, and of each pair of pustules which might be expected to coalesce later, was mapped on a label which was then attached to the leaf. In this way it was possible to tell, when the pustules became older, which pustules were simple, and which were compound. If the distance between a pair of pustules was greater than 4 mm., each of the components was considered as a simple pustule, as the two seldom achieved a thorough coalescence. Usually the points at which infections took place on the leaf were easily discernible, but sometimes two infections occurred so close together that it was extremely difficult to decide whether or not there were two infections. This was an ever-recurring difficulty. Where there was any doubt, the pustules were always classed as simple. For this reason certain pustules which were very probably compound were tabulated as simple pustules.

As the number of barberry plants available and suitable at any one time for inoculation purposes was limited, the inoculating was done at different

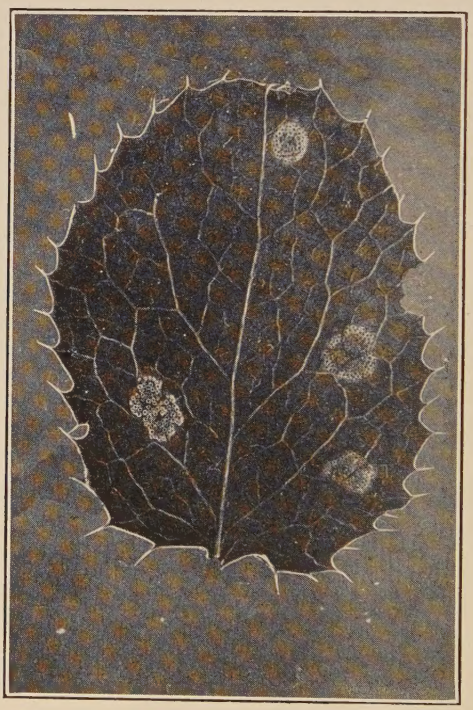

FIG. 1. Under side of a barberry leaf showing one compound pustule with aecia, on left of midrib, and three simple pustules without aecia, on right of midrib. Photographed 20 days after inoculation. ×2.

times. The data were recorded 20 days after each inoculation. Before the end of that time, the majority of the paired pustules, designated as compound, had completely coalesced, and where the paired pustules, or components, were of opposite sex, aecia had appeared. In a few of the compound pustules in which the components were relatively far apart, coalescence was not yet thoroughly accomplished. The results are summarized in table 1.

TABLE 1.—*Number of simple and compound pustules of Puccinia graminis on leaves of Berberis vulgaris in which aecia had developed and in which aecia had not developed 20 days after inoculation*

Kind of pustule	Total no. of pustules	No. of pustules	
		with aecia	without aecia
Simple (monosporidial in origin)	174	11	163
Compound (bisporidial in origin)	59	24	35

It should be mentioned that these data were collected before the function of the pycnia was discovered, and it is quite possible that, in at least some of the 11 simple pustules which gave rise to aecia, the development was induced by the transfer to them of nectar from pustules of opposite sex, by insects or other agencies. It is possible also that a few others, although tabulated as simple pustules, were really bisporidial in origin.

From time to time after the data recorded in table 1 were taken, some pustules of both types, which had hitherto produced no aecia, developed aecia. The appearance of aecia in a few of these compound pustules may be explained by the fact that the two components of each pustule, although of opposite sex, were at first rather widely separated (about 4 mm.), and consequently the interaction of their mycelia was delayed. The belated development of aecia which occurred in the other compound pustules and in the simple pustules was probably induced, at least to some extent, by a fortuitous transference to them of nectar from pustules of opposite sex. In some of the pustules aecia may have arisen spontaneously, but doubt is thrown on this possibility by the results obtained with simple pustules of *P. helianthi* recorded elsewhere in this paper.

Many of the pustules, both simple and compound, which did not produce aecia persisted for eight or nine weeks, some even for a longer time. When the experiment finally terminated, it was found that of the 174 simple pustules and the 59 compound pustules, 117 of the former and 23 of the latter had failed to develop aecia, a result which, after due allowance is made for the imperfections of the experiment, is fairly conclusive evidence of the heterothallic nature of this rust.

AECIA IN SIMPLE PUSTULES

When the effect of mixing the nectar of simple pustules of opposite sex was discovered, suspicion arose concerning the ability of such pustules to produce aecia spontaneously. In the early stages of the investigation, approximately 40 per cent of these pustules developed aecia, but later, when the plants bearing the pustules were kept in cages, aecia appeared in a very much smaller number. The inference was drawn that quite possibly, in the frequent examination of these pustules—the leaves were turned over by thumb and fore-finger, the nectar of (−) pustules was occasionally carried to (+) pustules, and *vice versa,* thus inducing the development of aecia in some pustules. Moreover, no protection was afforded these pustules against the visitation of flies or other insects which might by chance have entered the greenhouse.

In order to get more exact information as to whether simple pustules were capable in themselves of producing aecia, the following experiment was undertaken. Leaves of sunflower seedlings growing in the greenhouse were inoculated with sporidia of *Puccinia helianthi* when the two first true leaves were about one inch or slightly less in length. The sporidia were allowed to fall sparsely on to the upper surface of the leaves.

Usually about six seedlings grew in a pot, but, whenever the minute pustules appeared, each plant showing infection was transplanted alone in a separate pot and covered with a screen wire cage. It was thought that by keeping each plant in an individual cage the opportunity for the transfer of nectar by any insect which might enter the cage would be reduced to a minimum. In general, not more than one pustule occurred on any individual leaf. Indeed, many of the plants bore but a single pustule. Infrequently, however, two or more occurred on the same leaf. All uninfected leaves were removed from the plants and no new ones were allowed to develop.

With the exception of a few compound pustules which were readily discernible, and a small number of pustules which were marked as simple but which produced aecia within 12 or 14 days, all the others were apparently simple. Those which were at first marked as simple but developed aecia within two weeks from the time of inoculation possibly arose from two infections which took place so close together that the two infections appeared as one. Sometimes it was difficult to determine with certainty whether a single sporidum caused the infection, or whether it arose from two sporidia which alighted side by side on the leaf. It can not be said with confidence that these apparently simple pustules were in reality compound, but the likelihood is that they were. In order to give any compound pustules which might have had components of opposite sex an opportunity

to develop aecia and to avoid dealing with doubtful cases, no data were recorded until the pustules were 17 days old. There were altogether 228 pustules, all apparently simple ones, in which no aecia at all were evident. Of these, 93 were borne singly, one on each plant; of the remaining 135, usually two, but sometimes three or even four, occurred on the same plant. None of the pustules which developed on the cotyledons were considered, for the majority of the cotyledons became chlorotic and died comparatively early in the experiment.

Not all of the pustules persisted for the same length of time. Some became necrotic during the fourth week; most of them died before the end of the sixth week; but a few were still living and exuding nectar in their peripheral region when seven weeks old. The experiment terminated at the end of the seventh week. Within that time, aecia appeared in 11 of the 228 pustules. There was no regularity in their time of appearance, as if in response to a common stimulus. First one pustule—then a few days or a week later another pustule — would give rise to aecia, as if influenced by a separate and distinct stimulus. The results are summarized in table 2.

TABLE 2.—*Number of simple pustules of Puccinia helianthi, over 17 days old, which produced aecia and which did not produce aecia*

Pustules per plant	No. of pustules which produced	
	Aecia	No aecia
One	2	91
More than one	9	126
Total	11	217

From the time the pustules first appeared, an examination of each plant was made daily in order to destroy any insect which might have found its way through the mesh of the cages and thus gained access to the plant. White flies (*Aleyrodes*) and thrips (*Heliothrips*) were about the only ones which succeeded in doing this. Any of these that were found were removed and destroyed. The former were rarely ever seen in contact with the pustules, but the latter showed a decided preference for nectar as a diet. In spite, therefore, of considerable precautions, there was the possibility, especially in those cages in which the plants bore more than one pustule, that, during the seven weeks over which the experiment extended, some slight mixing of nectar took place.

Possibly, with the absolute exclusion of insects, or other means by which nectar might be transferred from one pustule to another, aecia would have arisen in none of these pustules. This, however, was not demonstrated.

At present, it is impossible to say that aecia do not arise spontaneously in pustules of monosporidial origin, but it has been clearly shown that, under the conditions of the experiment, only a very small percentage of such pustules did give rise to aecia. It is desirable that this point should be definitely settled, but to do so would require very rigid control and many more pustules than the writer can hope to have available this year.

OCCURRENCE UNDER NATURAL CONDITIONS OF PYCNIA UNACCOMPANIED BY AECIA

So far as the writer is aware, it is not recorded that pustules of monosporidial origin occur in nature, although undoubtedly they have been seen by other observers and mistaken by them for young pustules in which aecia had not yet developed.

From observations made during the summer of 1927 and of 1928, it has been found that in nature pycnia are frequently unaccompanied by aecia in the following rusts: *Puccinia graminis,* on *Berberis vulgaris* var. *purpurea*; *P. coronata* Cda., on *Rhamnus cathartica*; *P. pringsheimiana* Kleb., on *Ribes grossularia* (cultivated and wild); and a *Gymnosporangium* sp. (possibly *corniculans* Kern.), on *Amelanchier alnifolia.* Very probably the phenomenon occurs under natural conditions in other rusts also, but, as those mentioned were the most accessible, observations were confined mostly to them.

Leaves of these hosts bearing young and apparently simple pustules were marked by means of a small tag as soon as the pustules were noticed. When the pustules were about 14 or 15 days old, those which showed no evidence at all of aecia were selected for further observation. It was thought that if any of the first marked pustules originated from two contiguous infections and the mycelia of the two were of opposite sex, aecia would appear in such compound pustules within this time; if the mycelia were of the same sex, no aecia would be expected. In all the carefully examined pustules, pycnia were invariably present.

From time to time during the next three or four weeks, these pustules were examined. Within that time, some of the pustules produced aecia, but others did not. All of the pustules which produced aecia and most of those that produced none became necrotic, but a few of the latter persisted fresh-looking and vigorous for a week or more longer. The results of these observations for 1927 are given in table 3.

It should be noted that none of these pustules were protected in any way from the visitation of insects, and there is little doubt that through their agency the transfer of nectar from one pustule to another took place, as insects of various kinds were seen flying about or crawling over the leaves

TABLE 3.—*Summary of observations made in 1927 on the occurrence in nature of aecia in simple pustules*

Name of rust	No. of pustules 14 days old	Observation period (weeks)	No. of pustules at end of period	
			with aecia	without aecia
Puccinia graminis	50	4½	37	13
P. coronata	61	2½	45	15
P. pringsheimiana	60	3	16	44
Gymnosporangium sp.	60	3	52	8

of the host plants, some even in contact with the pustules. However, it should also be noted that, on account of the excessive precipitation, the pustules were frequently washed, and only very seldom was there any noticeable amount of nectar available for transfer. This fact accounts, perhaps, for the failure of so many of the pustules to produce aecia, despite the activity of the insects.

During the summer of 1928, young pustules which were apparently monosporous in origin were again marked, but instead of leaving them exposed, as was done in the previous year, most of the leaves bearing them were covered with one ply of white cheese-cloth, in order to intercept as far as possible the visitation of insects. This protection excluded fairly effectively most of the winged insects, but not so well, perhaps, ants and small spiders.

Not all the pustules marked were found as soon as they appeared. At the time the coverings were applied, some would be at least one week old; most of them, however, were just appearing, or not more than two or three days old. Unfortunately, the pustules available for study were much less numerous than one could have wished. Practically no infection occurred on *Rhamnus cathartica* at the Agricultural College, Winnipeg. None at all were found on *Amelanchier alnifolia*. Only a few appeared on *Berberis vulgaris*, and the pustules on *Ribes grossularia* were relatively scarce.

Certain of the pustules on *Ribes* were left unprotected in order to serve as a check for the protected ones, and for comparison with the data collected the previous year. The results are summarized in table 4.

It will be seen from tables 3 and 4 that a much smaller percentage of the covered pustules in 1928 produced aecia than of the uncovered ones in 1927; and that, in 1928, less than 20 per cent of the covered pustules of *P. pringsheimiana* developed aecia compared with 50 per cent of the ones which were not covered. Apparently, by preventing the free access of insects to the pustules, the opportunities for transferring the nectar of one

TABLE 4.—*Summary of observations made in 1928 on the occurrence in nature of aecia in simple pustules*

Name of rust	No. of pustules	Observation period (weeks)	No. of pustules at end of period	
			with aecia	without aecia
Puccinia graminis (covered)	20	3	4	16
P. coronata (do)	2	3	0	2
P. pringsheimiana (do)	33	3	6	27
do (uncovered)	18	3	6	12

pustule to another were reduced, and, consequently, the number of simple pustules which remained free of aecia was augmented. It is not probable that the cheese-cloth covering intercepted sufficient sunlight to inhibit or retard materially the formation of aecia. This supposition is supported by the fact that a certain number of the covered ones produced aecia. Very probably the development of aecia in these pustules was induced by the

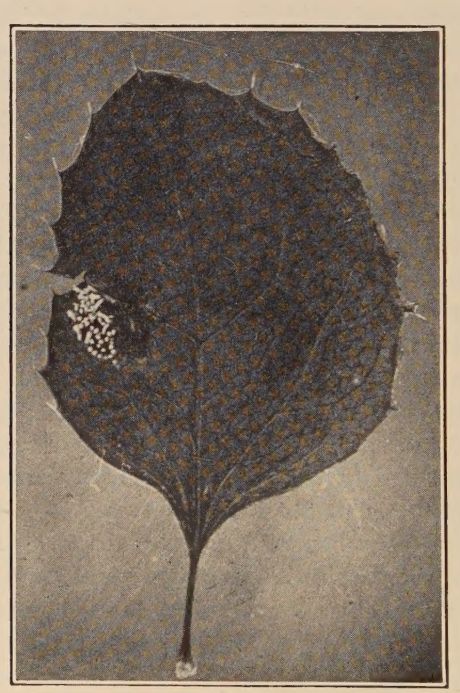

FIG. 2. Under side of a barberry leaf showing aecia present on the left-hand side, but not on the right-hand side, of a pustule of *P. graminis* which arose from an infection by natural inoculation. ×2.

transfer of nectar by insects before the covering was applied. This of course is but conjecture.

From the fact that simple pustules of *P. coronata,* of *P. pringsheimiana,* and of the *Gymnosporangium* sp. behave under natural conditions as do similar pustules of *P. graminis,* and as *P. graminis* has been shown by experiments in the greenhouse to be heterothallic, it may be inferred that these three rusts also are heterothallic.

While the data recorded above were being collected, it was noticed that in a few of the pustules which had not previously developed aecia, aecia began to appear at one side or in one section of a pustule. This phenomenon was observed in simple pustules of *P. coronata,* of *P. pringsheimiana,* and of *P. graminis* (Fig. 2). A plausible explanation of this behavior is that the nectar from pustules of opposite sex had been deposited on the upper surface of those sections only of the pustules, and as a result aecia had developed.

Using this interpretation as a cue, an attempt was made to reproduce similar results in the greenhouse in simple pustules of *P. helianthi.* The nectar from a dozen or more pustules of this rust was collected and deposited in one drop. By so doing it was hoped that some nectar would be gathered

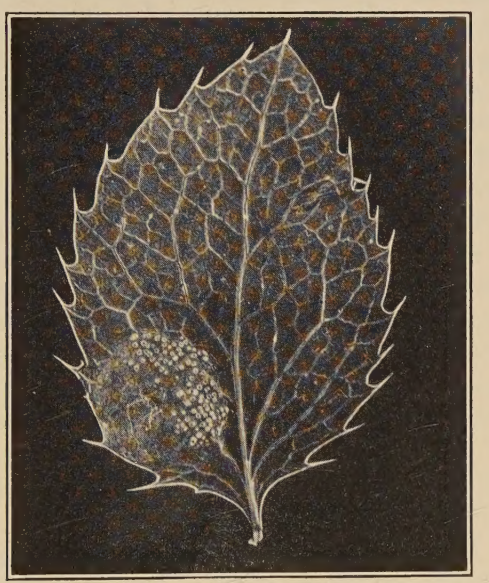

FIG. 3. Under side of a barberry leaf which was artificially inoculated in the greenhouse, showing aecia present on the right-hand side, but not on the left-hand side, of a simple pustule of *P. graminis.* When the pustule was 20 days old, a small amount of composite nectar was applied to the upper surface of that section which now bears the aecia. The photograph was taken 7 days after the nectar was applied. $\times 2\frac{1}{2}$.

from pustules of opposite sex and that the drop would contain both (+) and (−) pycniospores. Small amounts of this composite nectar were applied to one side only of the simple pustules when they were approximately four weeks old. In each case, aecia developed within four or five days on the under surface of the pustule directly beneath the section to which the nectar was applied. The experiment was repeated with simple pustules of *P. graminis,* three and one half weeks old, with similar results (Fig. 3).

The exudation of nectar is much more copious in pustules of *P. graminis* than in pustules of *P. helianthi.* Therefore, before the composite nectar was applied to any pustule of *P. graminis,* as much as possible of the nectar which it had itself produced was drawn off by means of a capillary tube. In most of the pustules the aecia remained localized, particularly in those of *P. helianthi*; but in some pustules of *P. graminis* there appeared to be a tendency for aecia, after they had formed in the treated section, to develop belatedly in the other part of the pustule—the course of development being from the treated section toward the opposite edge.

It is difficult to say to just what cause this belated formation of aecia was due. Possibly, in the course of a few days the pycniospores were carried slowly across the surfaces of the pustules by flowing movements of the nectar, owing to the fact that the pustules are usually convex or concave, rarely flat, and day by day as more nectar accumulated, adjustments in the level of the nectar took place, thus bringing the composite nectar into contact with pycnia in the untreated section of the pustule. This explanation seems to be supported by the fact that in pustules of *P. helianthi,* where, under greenhouse conditions, the amount of nectar exuded is comparatively small, aecial development was confined almost entirely to the sections treated. Another possible explanation for the development of belated aecia in the untreated sections of these pustules is that diploid mycelia may have gradually extended from the treated into the untreated parts and there given rise to aecia.

The occurrence in nature of aecia in one sector, or one part, of a simple pustule and the duplication of a similar phenomenon by experimental methods in the greenhouse are of interest in themselves, but they are mentioned chiefly to lend additional support to the evidence already given that *P. coronata* and *P. pringsheimiana* are heterothallic. In appearance and behavior they simulate the two rusts which have been shown by experimentation to be heterothallic.

SUMMARY

1. Experiments carried out in the greenhouse have shown that *Puccinia graminis* is heterothallic.

2. Experimentation has shown that, under greenhouse conditions, aecia develop spontaneously in few, if any, simple pustules of *Puccinia helianthi.*

3. Observations have shown that simple pustules of *Puccinia coronata,* of *P. pringsheimiana,* of a *Gymnosporangium* sp., and of *P. graminis* occur under natural conditions on their respective aecial hosts. This fact indicates that, in addition to *P. helianthi* and *P. graminis,* these three other rusts also are heterothallic.

4. Pustules of *P. coronata* and *P. pringsheimiana,* as well as of *P. graminis,* have been found in nature bearing aecia in only one sector, or part, of each pustule. Experimentation in the greenhouse with simple pustules of *P. graminis* and *P. helianthi* has reproduced similar conditions, a result which further indicates that *P. coronata* and *P. pringsheimiana* are heterothallic.

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